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INVESTIGATION OF THE POSSIBILITY FOR ENZYMATIC UTLIZATION OF CHICKEN BONES

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ABSTRACT

A perspective approach is proposed in which ground chicken bones remaining in the poultry slaughterhouses after extracting the meat from them for human food are treated by minimal amounts of purified alkaline protease (Alcalase). Using this process two principal fractions were obtained- ***soluble protein hydrolysate*** *(SPH) that can be used as component of microbiological cultivation broth media or as a "protein" nutritive additive in the diet of domestic animals and* ***insoluble mainly inorganic fraction*** *(IF) that can find application as fertilizer containing Ca, Mg, and small amounts of P, C, and N or can be used as additive in the diet of hens and other domestic animals. The yields of SPH and IF regarding the quantity of the starting chicken bones were about 22% and 18%, respectively. The process is perspective since: 1. It is energy saving especially when the SPH fraction is used directly for animal feeding without drying; 2. It is not labour consuming; and 3. A waste product is converted into useful substances without any pollution for the environment.*

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Introduction

The organic materials remaining as a result of the processing of hens in the poultry slaughterhouses create considerable problems even when it comes from animals that were bred specially for human consumption and are fully healthy. Since these unused materials contaminate the environment, the contemporary European legislation prescribes incineration (6) if a better solution is not found, for instance as additive to the food of wild animals. Burning of these thrown out materials, however, is accompanied with high expenses for transport, manpower, salaries, energy, appliances for incineration, etc. Besides, the environment is polluted with the carcinogenic dioxins, nitrogen oxides and other toxic substances.

Methods for additional treatment and utilization of the mentioned organic materials have previously been described (10, 11, 14, 15, 16, 17). Usually, these materials are firstly hydrolyzed with strong acids or alkalis at elevated temperatures. During such treatment, however, destructive processes take place and some amino acids are changed into non-physiological compounds. Other investigations proposed direct attack with microorganisms (9) which requires sterile conditions and special equipment. These facts made us to study the possibility for enzymatic degradation of milled bones.

In the present study an enzymatic method carried out at relatively low temperatures for degradation of milled hen bones, cartilages, tendons, skin, remaining of meat, etc. is described. Elevated temperatures are employed only for denaturation of the proteins and for drying of the final products.

Materials and Methods

Materials

A mixture of roughly depleted from the meat bones together with feet, necks, heads and other parts of the hens were used as starting material. Before the killing of the hens, their perfect health status was verified. Alcalase Novozymes for nutritive purposes was used as hydrolysing agent. All other reagents were of analytical grade.

Partial hydrolysis

The starting material was minced with a meat-chopping machine (Tefal LE HACHOIR 1800), supplied with a grid whose holes had a diameter of 4 mm. The obtained forcemeat was mixed with tap water (1:1.5) and boiled for one hour. After cooling to 50-55^{o}C, a mass that contains little liquid was obtained. Nevertheless, a strong mechanical stirrer was placed inside and a dilute solution of the proteolytic enzyme was added in a proportion of 15000 CTA proteolytic units (12) to 1 kg forcemeat. After about 30 min the primarily almost solid mixture starts to progressively become liquefied and this process continues for another one hour. The development of the process is followed by measuring the dry matter content in the liquid phase using a refractometer. Although the liquid phase is very turbid it is still possible to monitor the hydrolysis when strong artificial light is used. In **Table 1** are given exemplary kinetic data of the enzyme hydrolysis. It is seen that the main process of hydrolysis continues about one and a half

hour. Then the mixture is boiled for 10 min and subjected to filtration through a synthetic textile filter. The properly sucked out filter cake was transferred into stainless trays, dried at 80-100°C, milled to rough powder and stored in hermetically stoppered plastic containers. This material will be designated further as ***insoluble mainly inorganic fraction*** (**IF**). It can be used as such as fertilizer in horticulture or as additive in the diet of animals since it contains residual protein, some fat and inorganic ingredients as calcium and magnesium phosphate and carbonate. The yield of the IF-fraction depends on the degree of depleting the bones from the meat, the humidity of the starting material, the water content of the filter cake and the age of the hens but usually varies between 15 and 24% of the starting material. The liquid phase usually represents a high quality dense and almost clear bouillon possessing a dry matter between 12 and 16%. This fraction we called ***soluble protein hydrolysate*** (**SPH**). It could be spray-dried or gently boiled to 55-70% dry matter, bottled in sterile containers and stored in refrigerator, for longer time- better in a freezer at minus 15 to -20°C, or further processed in order to obtain more expensive products. Sometimes it shows some opalescence and then it could be clarified by the method of Tzokov et al. (18). Its yield also depends on the factors mentioned for the IF-fraction and varies between 19 and 26% when calculated as percent from the starting material and dry matter in the final product.

TABLE 1

Enzymatic degradation of minced chicken bones

Minutes from the beginning	Dry Matter	pH	Temperature
0	7.0%	6.4	50-53°C
15	9.0 %	6.5	54-55°C
30	10.5 %	6.6	55°C
60	11.0 %	6.6	55°C
90	11.5 %	6.6	55°C
120	12.0 %	6.6	55°C
150	12.0 %	6.6	55°C

The total quantity of peptides and amino acids in the final products was determined as "protein content" according to the method of Lowry (3, 7) and the biuret method (8). In both cases human serum albumin was used as standard.

The amino nitrogen content was determined according to the Adler-Nissen method (1), total carbohydrates- according to Dubois et al. (5) and the gel chromatographic analysis was performed as described by Andrews (2) on Sephadex G-25 and Sephadex G-75, both in 50 mM sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl. The registration was carried out at 206 nm in order to establish both peptides and free amino acids present in the sample.

The determination of protein and amino nitrogen content requires a dissolved sample. Since the IF contains insoluble in water and water-salt systems components it was extracted prior to analysis. For the determination of protein and amino nitrogen, samples of 5-10 mg of the dry IF were stirred with 1 ml 0.1 M NaOH for 15 min. The mixtures were centrifuged (10000 × g, 5 min) and the clear supernatants of base- soluble peptides were subjected to analysis.

The dry matter of the different solutions and gels was determined using an Abbe refractometer.

The ash content was determined by measuring about 1 g of sample (up to the 4th digit after the decimal point), incinerating the sample for 20 min at red light (about 600°C), cooling it in a desiccator over dry NaOH and measuring the residue.

Results and Discussion

In **Table 2** are given characteristics of the fractions obtained after the enzymatic hydrolysis of the ground chicken bones. Taking into account that the dry SPH-fraction contains 7-10% water and some salts one can conclude that its organic matter actually consists mainly of proteins and their degradation products obtained during the enzyme hydrolysis. Considering the last column of the table which represents the percent of soluble fractions in IF respective to those in SPH it is seen that they gravitate about 20% which shows that about 20% of the starting solution remains in the filter cake. This value is not valid for the carbohydrates maybe because of presence of some insoluble carbohydrates coming probably from the cartilages in the starting materials. Actually, during the described enzyme hydrolysis most of the cartilages disappear as such but some parts of them can be seen in the mixture before filtration.

TABLE 2

Protein, amino nitrogen, carbohydrate and ash content in SPH and IF

No.	Type of Analysis	SPH	IF	% SPH in IF
1	Protein acc. to (3, 7) in %	82	16	19.5
2	Protein acc. to (8) in %	66	14	21
3	Amino nitrogen acc. to (1) in %	1.2	0.24	20
4	Carbohydrates acc. to (5) in %	2.6	1.6	
5	Ash content in %	4.9	55.7	

In order to find the approximate molecular masses of the products obtained at the enzyme hydrolysis we subjected the SPH-fraction to gel chromatographic analysis. Two gels, namely Sephadex G-25 (**Fig. 1**) and Sephadex G-75 (**Fig. 2**) were used.

The conclusions that can be made from the gel chromatography analyses made on the two types of carriers are in agreement with each other. The predominant components of the soluble enzymatic fraction (SPH) consist of peptides with molecular masses between 20 and 1 kDa. About 20-30% belong to free amino acids and dipeptides.

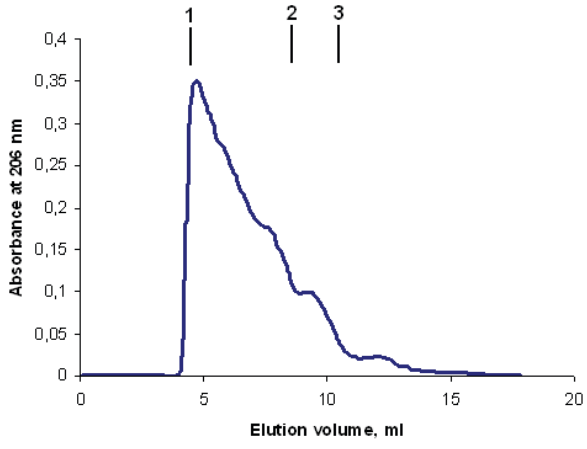

Fig. 1. Gelchromatograpy on Sephadex G-25. Column 0.4 x 32 cm, 26 ml/h. As standards were used Blue Dextran (1), Gly (2) and Tyr (3)

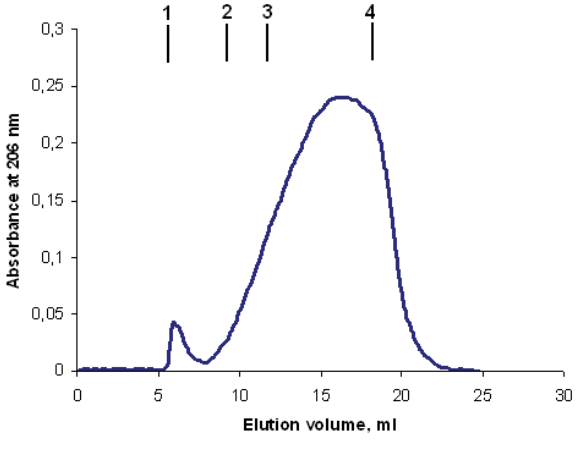

Fig. 2. Gelchromatograpy on Sephadex G-75. Column 0.4 x 34 cm, 15 ml/h. The following standars were used: 1. Human serum albumin- dimer; 2. Human serum albumin- monomer (69 kDa); 3. Soybean trypsin inhibitor (21 kDa); and 4. Gly

The SPH-fraction was used to replace the "Difco peptone" component in the standard microbiological broth medum of Kosmachev (13), namely 3.0% agar plates containing (g L^{-1}) peptone- 5; corn steep liquor- 5; starch- 10; NaCl- 5; and $CaCO_3$- 5. The following microorganisms were tested for their ability to grow and develop on these plates: *Thermoactinomyces* specia, *Bacillus subtilis*, *Bacillus mesentericus*, *Bacillus mycoides*, *Penicillium* species. The results read on the SPH-fraction plates did not differ from those read on the Difco-pepton agar plates. This fact shows that the SPH-fraction can find application in the laboratory and industrial microbiology as a cheap component- substitute for the expensive Difco-peptone.

Conclusions

The principal advantages of the proposed method are several:

1. No harmful chemicals are added to the waste material in order to convert it into useful products;
2. No special appliances are needed for its performance;
3. The cost of the enzyme needed for hydrolysis of one kg minced bones is neglectfully small - several euro-cents (between €0.07 and 0.08).
4. The separation of the liquid phase from the solids in the enzyme hydrolyzate is easy by using a bag (sack) made of thick synthetic matter placed over a porous frame. The filtration takes place spontaneously over night preferably in a cool place and does not need any observation.
5. The filter cake is thick and dense, contains small amounts of water and can be dried easily in metal trays without adhering on the drying surfaces because it contains a lot of insoluble materials and only small amounts of peptides.
6. The method gives no waste products since all materials produced by it can be used as fertilizers.
7. After the enzymatic treatment the bone particles become brittle since the main part of collagen in them is dissolved and the grinding of the final dry product (IF) turns easy.
8. The method is energy saving since the enzymatic hydrolysis takes place at relatively low temperatures (about 50-55°C), and the conservation of the final products is made by evaporation of small amounts of water.

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